National Toxicology Program
Toxicity Report Series
Number 46

NTP Technical Report on the Hepatotoxicity Studies of the Liver Carcinogen

Methapyrilene Hydrochloride

(CAS No. 135-23-9)

Administered in Feed to Male F344/N Rats

July 2000

U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Toxicity Study Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals.

These studies are designed and conducted to characterize and evaluate the toxicologic potential of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Toxicity Study Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's toxic potential.

Listings of all published NTP reports and ongoing studies are available from NTP Central Data Management, NIEHS, P.O. Box 12233, MD E1-02, Research Triangle Park, NC 27709 (919-541-3419). Other information about NTP studies is available at the NTP's World Wide Web site: http//ntp-server.niehs.nih.gov.

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July 2000

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PEER REVIEW

The draft report on the toxicity studies of methapyrilene hydrochloride was evaluated by the reviewers listed below. These reviewers serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determine if the design and conditions of these NTP studies are appropriate and ensure that the Toxicity Study Report presents the experimental results and conclusions fully and clearly.

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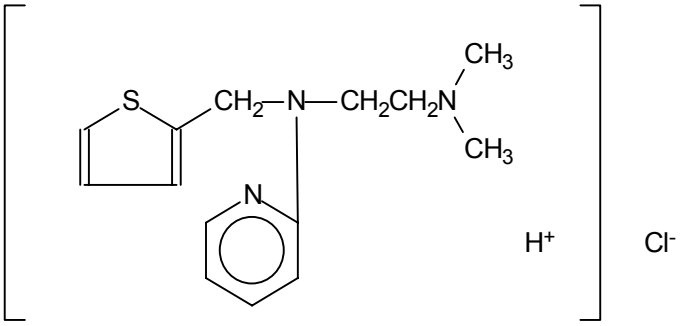
ABSTRACT

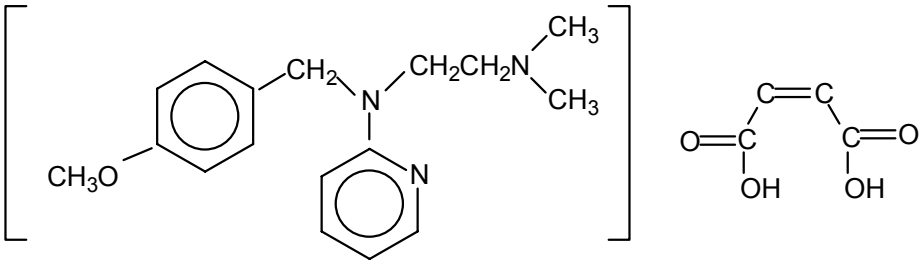


METHAPYRILENE HYDROCHLORIDE

CAS No. 135-23-9

Chemical Formula: $C_{14}H_{19}N_3S{\cdot}HCl$ Molecular Weight: 297.85

Synonyms: *N,N*-Dimethyl-*N′*-2-pyridinyl-*N′*-(2-thienylmethyl)-1,2-ethanediamine; 2-[(2-dimethyl-aminoethyl)-2-thenylamino]pyridine



PYRILAMINE MALEATE

CAS No. 59-33-6

Chemical Formula: $C_{17}H_{23}N_3O{\cdot}C_4H_4O_4$ Molecular Weight: 387.43

Synonyms: Paramal; paraminyl maleate; pymafed; pyra maleate; pyranilamine maleate; pyraninyl; renstamin; thylogen maleate
Trade Names: Anthisan; Dorantamin; Enrumay; Histan; Histatex; Histosol; Paraminyl Maleate; Stamine

Methapyrilene hydrochloride is a histamine H_1-receptor antagonist that was an active ingredient in many over-the-counter cold and allergy medications. In the mid- to late 1970s, studies in rats suggested that methapyrilene hydrochloride was a hepatocarcinogen, and the drug was removed from these preparations. In most cases, methapyrilene hydrochloride was replaced by pyrilamine maleate, a structurally similar analogue. As part of

a program to investigate mechanisms of toxicity whereby structurally similar chemicals produce different toxicities, these chemicals were studied for induction of cell proliferation and protein alterations by two-dimensional gel electrophoresis in the liver of F344/N rats. A complete toxicologic evaluation was not needed for this research-oriented study. Rather, the goal of the present study was to provide retrospective data from subchronic toxicity studies with the known rat carcinogen methapyriline hydrochloride that could then be used to predict the potential carcinogenicity of unknown chemical agents and that could also be compared with similar data on the structural analogue pyrilamine maleate. Pyrilamine maleate differs from methapyrilene hydrochloride in the substitution of the thienyl ring with a paramethoxyphenyl ring. Pyrilamine maleate has been shown to produce an equivocal increase in the incidences of liver neoplasms in rats in 2-year feed studies, but only at 2,000 ppm, indicating that its potency, if any, to produce neoplasms is much less than that of methapyriline hydrochloride. The hepatocarcinogenic peroxisome proliferator Wy-14,643 was included in this study as a positive control that is known to induce cell proliferation, as well as protein alterations, in the liver.

In the 14-week study of methapyrilene hydrochloride, groups of 40 male F344/N rats were given 0, 50, 100, 250, or 1,000 ppm methapyrilene hydrochloride, 1,000 ppm pyrilamine maleate (negative control), or 50 ppm Wy-14,643 (positive control) in feed. Rats in all groups were administered bromodeoxyuridine (BrdU) by osmotic minipump for the assessment of hepatocyte proliferation. Ten rats from each group were evaluated on days 15, 29, and 43 and at 14 weeks. At these times, samples of liver tissue were analyzed for evidence of cell proliferation via BrdU labeling and proliferating cell nuclear antigen (PCNA) labeling.

There were no exposure-related deaths. Low mean body weights were generally observed in the 1,000 ppm methapyriline hydrochloride group and in the positive control group. Final mean body weights and mean body weight gains of rats exposed to 1,000 ppm methapyrilene hydrochloride were significantly less than those of the untreated control group at all time points. The final mean body weights of rats in the positive control group were significantly less than those of the untreated control group for rats evaluated on days 29 and 43 and at week 14; the mean body weight gains of rats in the positive control group were significantly less than those of the untreated control group on day 29 and at week 14.

Feed consumption by rats exposed to 1,000 ppm methapyrilene hydrochloride was significantly less than that by the untreated control group throughout the study. The predominant clinical observation related to methapyrilene hydrochloride exposure was thinness in rats exposed to 1,000 ppm; this finding was first observed on day 29.

On days 29 and 43 and at 14 weeks, the absolute liver weights of rats exposed to 1,000 ppm methapyrilene hydrochloride were significantly less than those of the untreated control group. At all time points, the relative liver

weights of rats exposed to 1,000 ppm methapyrilene hydrochloride and the absolute and relative liver weights of positive control rats were significantly greater than those of the untreated control group. No significant differences in liver weights were observed between the negative and untreated control groups at any time point.

Hepatic lesions were observed predominantly in the 250 and 1,000 ppm methapyrilene hydrochloride groups and in the positive control group. The incidences of bile duct hyperplasia, hepatocyte necrosis, hepatocyte mitosis, and hepatocyte hypertrophy in rats in the 1,000 ppm group were significantly greater than those in the untreated control group at all time points. The severities of hepatocyte hypertrophy and hepatocyte mitosis in 1,000 ppm rats were generally mild to moderate; the lesions occurring in 250 ppm animals were less severe. At each time point, the incidence of bile duct hyperplasia in 250 ppm rats was significantly greater than that in the untreated control group. The incidences of hepatocyte mitosis on days 15 and 29 and the incidences of hepatocyte necrosis on days 29 and 43 in rats in the 250 ppm group were significantly greater than those in the untreated control group. Incidences of pigmentation in the 250 and 1,000 ppm methapyrilene hydrochloride groups were significantly greater than those in the untreated control group on days 29 and 43 and at 14 weeks.

In the positive control group, the incidences of granulomatous inflammation were significantly greater than those in the untreated control group on days 15, 29, and 43. The incidences of hepatocyte hypertrophy and hepatocyte mitosis in the positive control group were significantly greater than those in the untreated control group on days 15, 29, and 43. The incidence of hepatocyte hypertrophy was also significantly increased in the positive control group at 14 weeks. The severity of hepatocyte hypertrophy in the 1,000 ppm methapyrilene hydrochloride group was generally greater than that in the positive control group at each time point.

In general, methapyriline hydrochloride produced a dramatic and sustained increase in hepatic cell proliferation over 14 weeks, whereas pyrilamine maleate at the same concentration produced few if any effects. Wy-14,643 also induced a large increase in cell proliferation which declined over time, as has been observed in previous studies.

The mean BrdU labeling indexes of the 250 and 1,000 ppm methapyrilene hydrochloride groups were generally significantly greater than those of the untreated controls at all time points. In the negative control group, the BrdU labeling index was significantly less than that of the untreated control group on day 29. The BrdU labeling index in the positive control group was significantly greater than that of the untreated control group at all time points.

On day 43 and at week 14, the mean PCNA labeling indexes of the 1,000 ppm methapyrilene hydrochloride group were significantly greater than those of the untreated control group. The mean PCNA labeling indexes of the

negative control group were significantly less than those of the untreated control group on days 29 and 43. On day 29, the mean PCNA labeling index of the positive control group was significantly greater than that of the untreated control group.

The mitotic indexes of the 1,000 ppm methapyrilene hydrochloride group were significantly greater than those of the untreated control group at all time points. The mitotic indexes of the 250 ppm group were significantly greater than those of the untreated control group on day 43 and at week 14.

At least 32 proteins underwent significant abundance changes at the highest exposure concentration of methapyrilene hydrochloride, and 39 protein changes were observed in the positive control group. Many, but not all, of the protein changes in the methapyrilene hydrochloride-exposed animals also occurred in the positive control group. Treatment with pyrilamine maleate produced no significant quantitative protein changes, as judged by the same criteria used for methapyrilene hydrochloride and Wy-14,643. Methapyrilene hydrochloride produced covalent modification of mitochondrial proteins as measured by the charge modification index. PCNA abundance in liver samples from the 250 and 1,000 ppm methapyrilene hydrochloride exposure groups on day 43 was significantly greater than that of the untreated control group.

Results of tests for induction of mutagenicity by methapyrilene hydrochloride were negative in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and in L5178Y mouse lymphoma cells, with and without S9 metabolic activation. However, positive responses were obtained in cytogenetic tests with cultured Chinese hamster ovary cells, in which methapyrilene hydrochloride induced sister chromatid exchanges and chromosomal aberrations. The increases in sister chromosome exchanges were obtained with and without S9, but chromosomal aberrations were increased only in the presence of S9.

In summary, the significance of the increased hepatic cell proliferation and the protein alterations observed in this study is not definite, but may be of predictive value for assessing the toxicity and carcinogenicity of chemicals in preclinical assays. A chemical which does not produce an increase in cell proliferation or a large number of protein changes may be considered safer than a similar chemical that produces many such changes.